ZOOLOGICAL SCIENCE 6: 817–831 (1989)

REVIEW

Nervous Organization of the Pineal Complex in Lower Vertebrates

Manfred Ueck[1], Kenjiro Wake[2] and Hideshi Kobayashi[3]

Institute of Anatomy and Cytobiology, Justus Liebig University, 63 Giessen, FRG, [2]Department of Anatomy, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113, [3]Research Laboratory, Zenyaku Kogyo Co. Ltd., Nerima-ku, Tokyo 178, Japan

ABSTRACT—The morphological and electrophysiological aspects of the neuronal organization of the pineal complex are reviewed in fishes and amphibians. In the frog pineal organ, two types of intrapineal neurons different in size and shape are distinguished using the AChE and NADPH-diaphorase methods: multipolar cells (the first cell type) interpreted as interneurons which receive inputs from a large number of photosensitive pinealocytes and transduce their responses to pseudounipolar cells (the second cell type) which send their axons to the brain. From this morphological perspective, the chromatic and achromatic responses obtained in the frog pineal complex are discussed. The distribution pattern of different types of nerve cells demonstrated by the AChE-method in the fish pineal complex varies widely not only between species but also regionally within the same pineal organ. Some unpublished results obtained from species which have not been investigated till now confirmed previous observations. In accordance with the results obtained by others in the retina, where more than twenty different types of amacrine cells and more than ten types of ganglion cells have been identified, our results indicate subpopulations of nerve cells in the fish pineal complex. Further investigations are necessary to complete our knowledge about the nervous organization in the fish pineal complex: this would be a precondition for a better understanding of the electrophysiological results.

INTRODUCTION

The pineal complex of non-mammalian vertebrates develops intracranially as a diencephalic evagination; additionally, in some fish species a parapineal organ exists [1–5] and in some anuran and reptilian species, a shift of part of the pineal anlage into the skin (frontal organ of the frog) or into a hole of the skull (parietal eye of the lizard) in observed [see 6–9]. The pineal complex is photosensitive and contains photoreceptive pinealocytes interconnected with pineal nerve cells [see 10]. Light perception is not involved in vision; light-dependent impulses transduced by pinealofugal (afferent) fibres to di- and mesencephalic areas are involved in the entrainment of circadian and circannual pacemakers for the synchronization of rhythmic body functions (such as skin pigmentation, phototaxis, orientation, locomotory activity, metabolic and thermoregulatory responses and reproductive cycles) with the photoperiod of the environment. Direct light perception may also be involved in the regulation of melatonin synthesis in the pineal organ of lower vertebrates.

This short review deals mainly with the nervous organization of the pineal complex in fishes and amphibians. There is great variety of morphology

Received June 19, 1989
[1] To whom reprint requests should be addressed.
This review was written during the stay of MU as a Research Professor at the Tokyo Medical and Dental University.

and ultrastructure of the pineal complex between species of different vertebrate classes, and also within the same vertebrate class. Extensive morphological studies have been made on the frog pineal complex; therefore these results will be reviewed first and discussed with electrophysiological findings. In the second part morphological and electrophysiological data of the fish pineal complex will be reported including some of our unpublished results.

I. The nervous organization of the frog pineal complex

The frog pineal complex consists of the frontal organ and the pineal organ. The frontal organ is localized in the subcutis of the skin, the pineal organ is located intracranially and these organs are connected by the frontal nerve. A pineal tract leaves the dorsocaudal part of the pineal organ toward the brain [see 11]. The pineal parenchyma consists of pinealocytes, glial cells and nerve cells. The pinealocytes are photosensitive and possesses outer segment structures resembling retinal cones, but they are shorter in length because of a smaller number of disks [12–14]. The inner and outer segments of the pinealocytes protrude inversely and eversely directed into the pineal lumen, which is an outpocketing of the third ventricle. The pinealocytes are in synaptic ribbon contact with intrapineal nerve cells [11–14].

The visualization of the distribution pattern of pineal nerve cells and the demonstration of different types of nerve cells have been difficult for a long time, because a) unlike the retina the nervous organization of the pineal nerve cells in not in parallel layers and b) the silver impregnation technique successfully used in the retina does not work in the pineal organ. Pineal nerve cells are shown using methylene blue staining [15, 16], but a better understanding of the neural organization in the pineal complex was attained when an acetylcholinesterase (AChE) technique was histochemically applied. [17, 18]. Approximately 60 nerve cells in the frontal organ and 220–320 nerve cells in the pineal organ are counted. The dorsal wall of the pineal organ, where the pineal tract arises, contains three times more nerve cells than the ventral wall. The nerve cells in the rostral part are bigger in size than in the caudal part. Two different types of nerve cells are observed: 1) big multipolar cells, localized more centrally in the frontal organ and within the parenchyma of the pineal organ, send their processes to different areas of the organ; they may be interneurons, and 2) smaller nerve cells with the appearance of pseudounipolar cells which send one process into the pineal tract. The ratio of the multipolar cells to the pseudounipolar cells is 1:4 for the frontal organ and 3:5 for the pineal organ. Additionally, 30–50 unipolar or pseudounipolar cells are clustered in juxtaposition with the pineal tract at the caudal end of the pineal organ, and another 30–50 neurons are scattered along the basis of the subcommissural organ. Some of these nerve cells each send a process toward the brain, but other neurons each send a process in the opposite direction, rostrally into the caudal part of the pineal organ via the pineal tract.

Electrophysiological studies of the pineal system in lower vertebrates have shown light-modulated electrical activity [see 19, 20]. A spontaneous activity of pineal nerve cells occurs in darkness and light changes the frequencies of this discharge. Constant illumination decreases the activity almost linearly with the logarithm of light intensity; a wide range of light intensity provides messages to the brain [21, 22]. The following two types of response are recorded using light flashes:

1) The chromatic response which consists of an opposed colour mechanism, i.e. an inhibition upon illumination by stimuli of short wavelengths and an excitation in response to light of longer wavelength. The net output depends on the balance between opposite inhibitory and excitatory processes in response to the particular spectral composition of the incident light [23]. The daily changes in spectral composition of light shift the chromatic response to another state of activation. It has been hypothesized that the nervous mechanism of such interactions in the pineal may be realized by two different receptor populations making synaptic contacts with a ganglion cell using inhibitory and excitatory transmitters [24]; another possibility would be that functionally polarized interneurons transfer information from one cone system to another [25].

2) The achromatic response which is characterized by a decrease of the firing rate or complete inhibition of the maintained discharge by light of all wavelengths; it is the most common response of the pineal. The achromatic response differs considerably from the chromatic response with respect to the absolute threshold, spectral sensitivity and the adaptation process. The lowermost light threshold of the achromatic response is of the same order as the light threshold of the frog retina; this indicates a highly developed nervous organization with a high degree of convergence of numerous sensory cells to one nerve cell [26].

A visual pigment with λ max 550–580 nm has been identified in the frontal organ [27] which is close to the sensitivity maximum of the achromatic response of pineal nerve fibres and, additionally, a visual pigment 502 in the pineal organ which is similar to that of accessory cones of the frog retina. A spectrosensitivity has been found to match the absorption spectrum of rhodopsin in the dark adapted pineal organ and of iodopsin in the light adapted pineal organ [28].

Illumination of the pineal complex of the frog evokes slow (graded) potential changes in addition to the spike activity of ganglion cells. The slow potentials may arise from ganglion cells as summated postsynaptic potentials or from photoreceptor cells as summated extracellular currents [29].

Pineal photoreceptors respond to light with a hyperpolarization of the membrane potentials as retinal photoreceptors do; this results in ganglion cell hyperpolarization and lowered spike activity. The depolarization of pineal photoreceptors in darkness results (as in the retina) in a continuous release of an excitatory transmitter, possibly L-glutamate or L-aspartate, from the pinealocytes to second-order neurons which cause a significant increase of neuronal cell firing. Taurine, by far the most abundant amino acid in pineal tissues, markedly decreased the spontaneous activity in half of the neurons tested, the remaining cells being unresponsive [30]. Almost all pineal neurons are inhibited by γ-aminobutyric acid (GABA); the GABA-induced inhibition interferes with the light-evoked inhibition of the ganglion cell activity, i.e. light reduced the strength of inhibition and shortened the effect of GABA. A major role of GABAergic mechanisms in the ganglion cell output of pineal neurons is suggested [31]. In the retina GABA seems to be a neurotransmitter in H1 cone horizontal cells (goldfish) and in a class of amacrine cells (mammals) [32].

More recently morphological results have been published indicating a more complex photosensitive pineal system. On the basis of ultrastructural characteristics and of histochemical and immunohistochemical findings, three types of photoreceptor cells have been distinguished in the frog pineal complex [33]. Using an antiserum raised against the opsin of bovine rods [34, 35] the outer segments of one population of pinealocytes showed a positive reaction; they are rod-type. This has been an unexpected finding that cone-like outer segments reacted with antisera against rod pigment. However, the ultrastructure of cone outer segments characterized by a decreased number of membrane disks and a large surface toward the extracellular space is a less differentiated form of photoreceptor membrane multiplication and has nothing to do with type of the photopigment present therein [36]. The other pinealocytes are characterized by rhodopsin immunonegative outer segments; they are subdivided into two populations of cone-type pinealocytes: one cone-type contains a lipid droplet in its inner segment, the second cone-type pinealocyte possesses a small inner and outer segment, an electron-lucent perikaryon and a spherule-like axon terminal [33]. Demonstrating Ca^{++}-ATPase activity according to the histochemical and cytochemical technique of Ando *et al.* [37] the opsin-positive outer segments (rod-type) contain Ca^{++}-ATPase activity, the opsin-negative outer segments of cone-type pinealocytes containing a lipid droplet in their inner segment are Ca^{++}-ATPase negative [38].

Photosensitive pinealocytes differ also in the shape of their perikarya: slender-type (rod-like), spherical-type (cone-like) and double cone-type pinealocytes can be distinguished after staining with the NADPH-diaphorase technique according to Scherer-Singler [39], [40, 41]. Also the basal end-feet show differences in their length, distal ramification and in the number of boutons which are single, bouquet-like and cluster-like [40]. The ramified pinealocytes can contact several second-

order neurons, as Boycott [42] has shown for some cones of the retina.

The NADPH-diaphorase reaction demonstrates a fourth type of pinealocyte scattered in the basal part of the parenchyma; these cells obviously have no contact with the pineal lumen [40]. Pinealocytes with a reduced photosensitivity and a more pronounced secretory function seem to exist also in the frog pineal organ [40, 41], similarly to those described in the lamprey [43]. A fifth type of pinealocyte which sends a long axon-like process into the pineal tract has been described in fish pineal organ [44].

The ribbon synaptic contacts that exist between neighbouring photosensitive pinealocytes and between pinealocytes and neurons should also be taken into consideration [45]. Thus, the different types of photosensitive pinealocytes and their interrelationships should be taken into account when chromatic and achromatic responses are discussed on the basis of morphological findings.

The intrapineal neurons, described in detail with the AChE method [18], have recently been reinvestigated with tracer techniques and with the NADPH-diaphorase method (Fig. 1) and the results are interpretated differently. Eldred and Nolte [45] labeled not only the pseudounipolar cells but also the multipolar neurons by retrograde transport of either cobalt or horseradish peroxidase through the frontal nerve. They concluded from their results that both types of nerve cells in the frontal organ send an axon via the frontal nerve to the brain. This result has been confirmed in other species of lower vertebrates [46]; the conclusion of the result led to diagrams of the nervous organization of the pineal complex in lower vertebrates which did not take interneurons into consideration [45]. Here, the achromatic and chromatic response of the pineal organ is explained as the result of an interaction between different types of photoreceptors and intrinsic secondary neurons [36].

The results obtained with the NADPH-diaphorase technique demonstrate clearly two different types of nerve cells [40, 47] and confirm the previous assumption of Wake *et al.* [18] of the existence of interneurons. The suggestion made on the basis of electrophysiological results, that the lowermost light threshold of the achromatic response indicates a highly developed nervous organization with a high degree of convergence of numerous sensory cells to one nerve cell [26, 48], is confirmed by the demonstration of big multipolar nerve cells, especially in the ventral wall of the organ, with the NADPH-diaphorase activity. These multipolar nerve cells send long processes, well documented in total preparations, in lateromedial and rostrocaudal directions ramifying and terminating in the so-called plexiform areas [40]. Plexiform areas are ultrastructurally well documented regions where terminals of pinealocytes and nerve cells are concentrated and in synaptic ribbon contact [11, 13, 14]. The NADPH-diaphorase positive end-feet of pinealocytes ter-

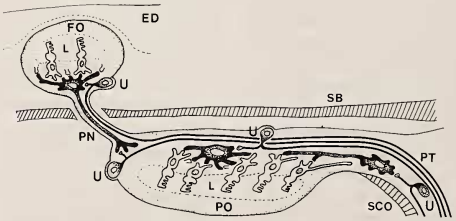

Fig. 1. Schematic drawing of characteristics of pineal nerve cells according to results obtained with the AChE-method and with the NADPH-diaphorase method. Multipolar nerve cells (black) are drawn as interneurons with relatively long processes; pseudounipolar cells (U) of the frontal organ (FO) and pineal organ (PO) send their axons to the brain. ED, epidermis; L, lumen; PN, pineal (frontal) nerve; PT, pineal tract; SB, cranial bone; SCO, subcommissural organ.

minate along the processes of the multipolar cells and in the plexiform areas at the ramified processes of the multipolar cells. Each multipolar cell sends its processes to different plexiform areas and the processes of several multipolar cells converge in each plexiform area. One multipolar cell is postsynaptic to synaptic ribbon synapses of a large number of pinealocytes in different areas of the organ. The pseudounipolar cells are localized adjacent to the plexiform areas; their perikarya and their axons which build up the pineal tract are not in contact with the end-feet of pinealocytes. Conventional synapses shown with an electron microscope and knobs of nerve fibres terminating at these pseudounipolar cells shown by silver impregnation techniques (Ueck and Ohba, unpubl. data) may indicate a presynaptic position of the multipolar cells to the pseudounipolar cells. Like the horizontal cells of the retina, the multipolar cells of the pineal complex receive information from a large number of photosensitive pinealocytes and they seem to transmit it to pseudounipolar cells in different plexiform areas. According to light microscopical findings, two types of multipolar cells may exist: in the first type perikarya and all the processes of these multipolar nerve cells are contacted by boutons of photoreceptor cells; in the second type of multipolar cells the perikarya and the proximal part of the processes are free of synaptic contacts and only distally a bouquet of boutons of pinealocytes contact the processes of the nerve cells. Therefore, each pseudounipolar cell can get information from several interneuronal multipolar cells terminating in the same plexiform area, and these multipolar cells may be of different types. In comparison with the retina which contains more than 20 types of amacrine cells and 10 types of third ganglion cells [49], the existence of subpopulations of multipolar cells and pseudounipolar cells in the pineal complex seems to be likely. Ekstroem *et al.* [50] described GABA-positive interneurons, and, additionally, GABA-positive neurons which send their axons to the brain in the pineal organ of the rainbow trout.

From the results of the study with NADPH-diaphorase activity [40, 41] it became clear that plexiform areas are important functional units of the pineal complex. The biggest plexiform area exists at the rostral tip of the pineal organ. Using silver impregnation techniques (Ueck and Ohba, unpubl. data) a subdivision of the frontal nerve into three bundles was found before it enters the rostral tip of the pineal organ: the two lateral bundles run into the right and left ventrolateral wall of the pineal organ, the median bundle enters the pineal in its rostromedian part and some of its fibers ramify and terminate in the previously mentioned big plexiform area. From this result it is thought that processes of multipolar cells, localized within the frontal organ, reach the most rostrally localized plexiform area via the frontal nerve. This conclusion would explain the retrograde filling of multipolar cells with horseradish peroxidase in the frontal organ via the frontal nerve, but also confirm the multipolar cells as interneurons. Pineal multipolar cells with one long process beside shorter dendrites would resemble retinal horizontal cells (B-type) with an axon (telodendrite) which ends in an "axon terminal system". On the other hand, the telodendrite of retinal horizontal cells is in synaptic contact with rods, the other dendrites of these cells contact with cones; each cone synapses with 3 or 4 horizontal cells of A-type and B-type; A-type and B-type horizontal cells are unspecifically connected with all populations of cones. The "axon terminal system" of telodendrites of retinal horizontal cells, which are in contact with rods, is electrically independent of the rest of the horizontal cells [42]. The demonstration of rod-like and cone-like photosensitive pinealocytes in the frog pineal complex [33, 36] and the demonstration of the existence of a telodendritic process of pineal interneurons (multipolar cells) [40] is an interesting aspect for the discussion of the chromatic and achromatic responses.

Two results obtained with the AChE method [18] have been difficult to explain, but can now be discussed following the results obtained by the NADPH-diaphorase technique [40, 47]: 1) The dorsal wall of the frog pineal organ contains three times more nerve cells than the ventral wall. In total preparations, the NADPH-diaphorase method, which stains both cell types, pinealocytes and pineal nerve cells, demonstrates a greater number of pinealocytes by area in the pineal organ

in the ventral wall than in the dorsal wall. This means that a greater number of pinealocytes is correlated with a smaller number of nerve cells in the ventral wall in comparison with the dorsal wall (Fig. 2). But the large size of multipolar nerve cells, 20–30 μm in diameter, and their spreading of dendrites contacting a large number of pinealocytes is not so pronounced in the dorsal wall than in the ventral wall. Conversely, the number of pseudounipolar cells adjacent to the plexiform areas is much larger in the dorsal wall, where the pineal tract arises, than in the ventral wall. This difference in the nervous organization of the dorsal and ventral wall is an additional indirect indication of the different functions of multipolar and pseudounipolar cells. 2) Further, as described above, some AChE-positive nerve cells, scattered around the pineal tract caudally to the pineal organ, send their processes toward the brain in the same way as the pseudounipolar cells, but other nerve cells send their processes in the opposite direction, via the pineal tract into the pineal organ. The latter may be interneurons, contacting not only photosensitive pinealocytes in the pineal organ, but also transmitting information outside the pineal organ to adjacent pseudounipolar cells.

II. The nervous organization of the fish pineal organ

The morphological characteristics of the fish pineal complex reveal striking differences between different species [4, 6]. The AChE method did not give such a clear piture of the nervous organization as it did in the frog [18]. The number, shape and distribution patterns of the intrapineal nerve cells vary widely, not only between different species [4], but also regionally within the same pineal organ. This structural variety makes it difficult to present a concept about the nervous organization at the moment. Therefore, published results are briefly referred to and our results obtained from species not previously described will be added (Figs. 3–6).

Wake [51] distinguished two different types of nerve cells on the basis of their size, intensity of AChE-activity and distribution pattern in the pineal organ of the goldfish, *Carassius auratus*: 1) large nerve cells with an extensive neuropil forma-

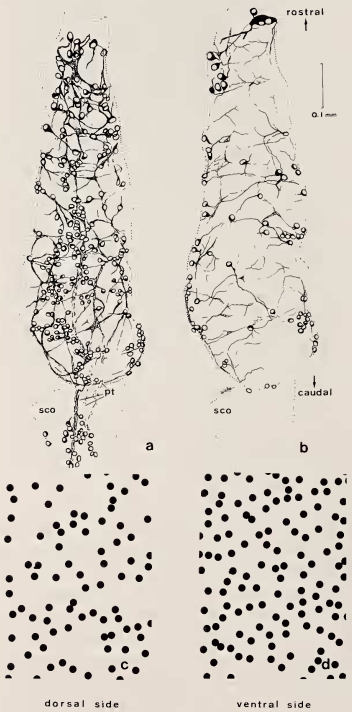

Fig. 2. a and b, Distribution of AchE-positive nerve cells in the dorsal (a) and ventral (b) walls of the pineal organ of *Rana ridibunda* [18]. The dorsal wall is more abundant in nerve cells than the ventral wall. A group of nerve cells is found in the area of the subcommissural organ (SCO); pt, pineal tract. c and d, Schematic drawing of the distribution patterns of the pinealocytes in the dorsal (c) and ventral (d) walls of the pineal organ; the number of pinealocytes by area is larger in the ventral (d) than in the dorsal wall (c). Drawings c and d were made from photographs with a final enlargement of ×1100, which were taken from an *in toto* preparation of the pineal organ after histochemical visualization of the NADPH-diaphorase activity.

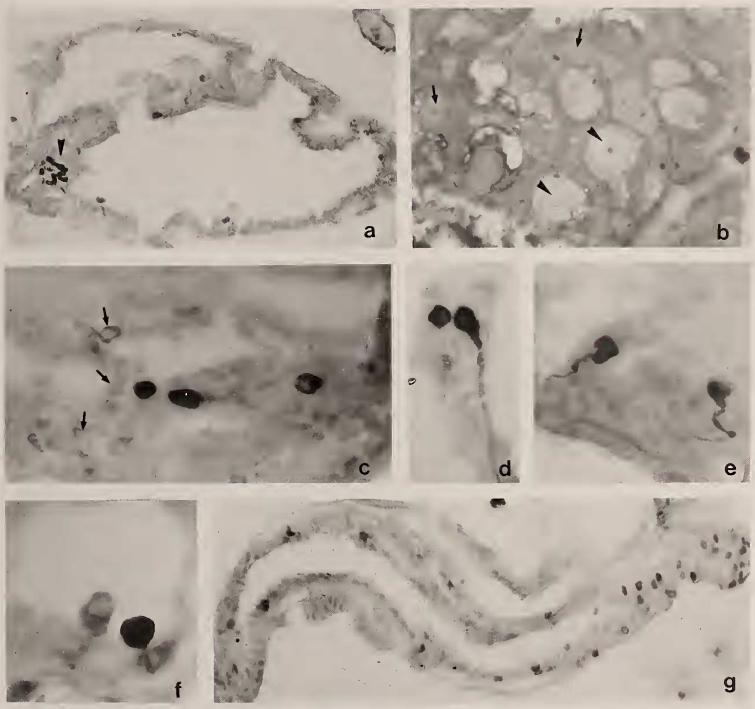

FIG. 3. *Fugu niphobles.* a, Ganglion-like concentration (▲) of intensely stained AChE-positive nerve cells in the medio-rostral part of the pineal end-vesicle. ×120. b, Ganglion-like area in a semithin section. Large nerve cells with a spherical nucleus (▲) and smaller nerve cells with an oval nucleus (⬆) are accumulated. ×1200. c, In some regions of the pineal end-vesicle small faintly stained nerve cells (⬆) can be recognized beside the bigger intensely stained nerve cells. ×490. d-f, The large intensely stained nerve cells in the pineal end-vesicle possess a long axon-like process; their perikarya are intraparenchymal or protrude into the pineal lumen (f). d, e, f, ×490. g, Pineal stalk; the number of intensely stained nerve cells by area increases in disto-proximal direction; the size of the nerve cells is smaller than in the pineal end-vesicle. g, ×240.

tion, demonstrating a moderate AChE activity only in the rostro-lateral regions of the hammer-shaped pineal end-vesicle, and 2) intensely stained, small nerve cells located in the medio-rostral area of the pineal end-vesicle and along the entire length of the pineal stalk.

Additionally, faint staining of the inner segments of the photosensitive pinealocytes made possible the estimation of the ratio of pinealocytes to nerve cells: approximately 50 pinealocytes are related to one large nerve cell in the pineal end-vesicle, and 3 to 4 pinealocytes to one AChE-positive nerve cell in the pineal stalk. Therefore, the size of the nerve cells is correlated with the number of pinealocytes in the surrounding area; however, it cannot be deduced that there are

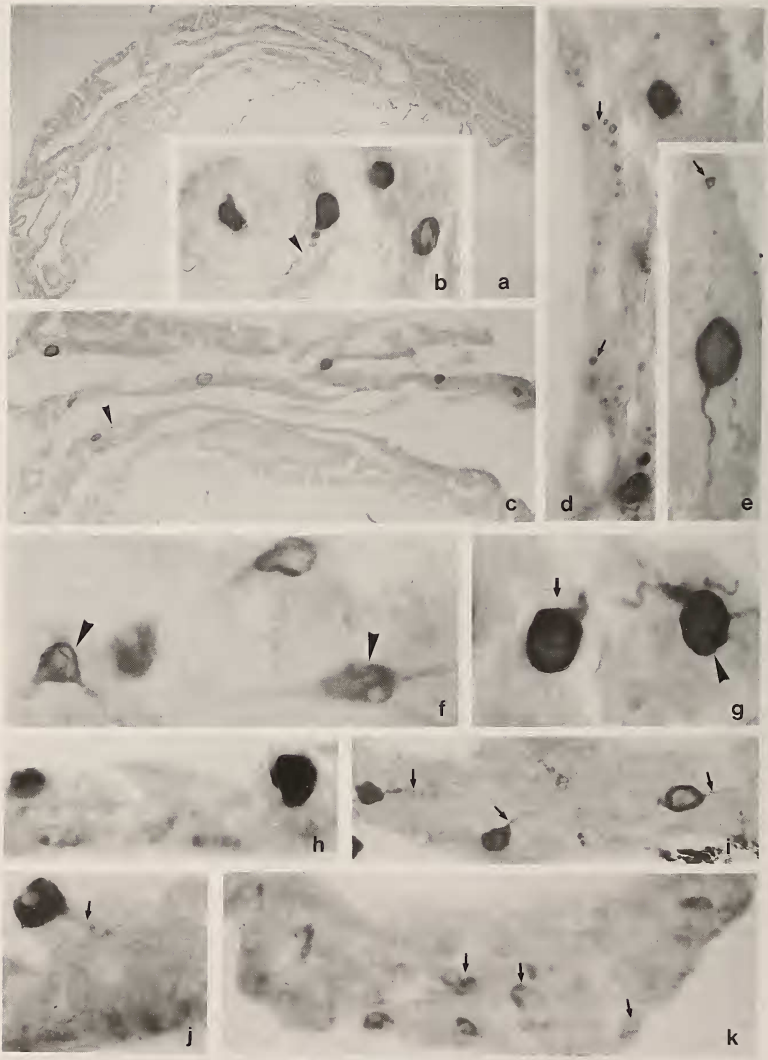

Fig. 4. (legends p. 827)

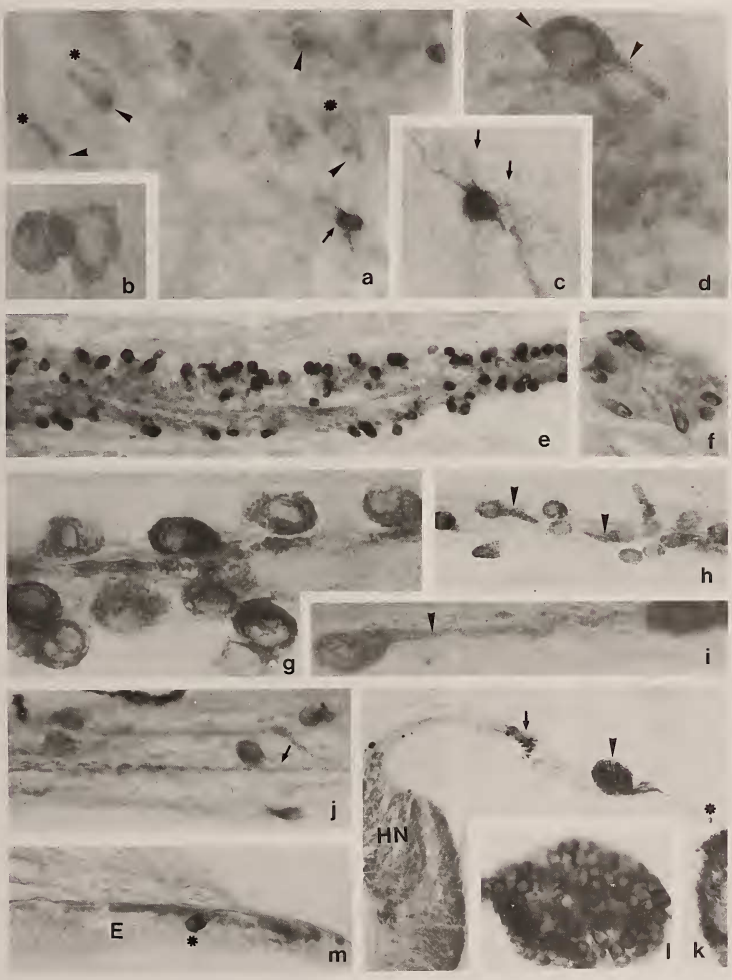

FIG. 5. (legends p. 827)

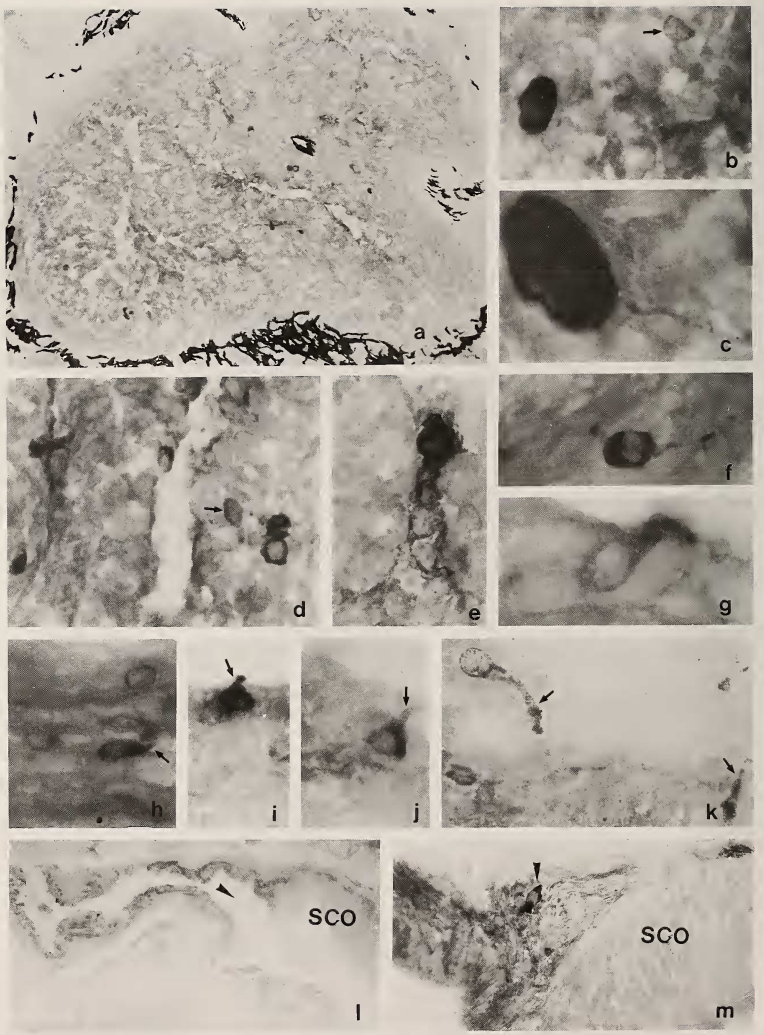

Fig. 6. (legends p. 827)

not—especially in the stalk area—two different types of AChE-positive nerve cells of similar size, because only the perikarya and not the cell processes are stained. Axons of pineal nerve cells are stained with silver impregnation technique [52]: long processes of nerve cells in the rostral part of the hammer-shaped pineal organ converge in a mediolateral direction into one lateral area on each side, an area where large bi- or multipolar cells are concentrated in the AChE-stained preparations. These two ganglion-like areas resemble the rostromedial ganglion described in the trout [2], in the rostro-ventral portion of the pineal end-vesicle of the minnow, *Phoxinus phoxinus* [53] and in *Fugu niphobles* (unpublished data) (Fig. 3a, b). In other fish species like *Fugu pardale* (Fig. 4a-e), *Scomber japonica* (Fig. 4f-k), the deep-sea fish *Helicolenus hilgendorfi* (Fig. 5a-m) and the shark *Triakis scyllia* (Fig. 6a-m), no ganglion-like accumulation of nerve cells exists, but large multipolar cells are scattered throughout the pineal end-vesicle. These large nerve cells are only in some areas surrounded by small nerve cells due to either difficulties in staining or to real regional differences (Figs. 3c; 4d, e, h, j). The large intraparenchymal cells may be functionally comparable to the multipolar cells of the frog pineal complex [40, 41] (Figs. 3d-f; 4b, e, i, j; 5a; 6b, c-unipolar), (Fig. 6f-bipolar), (Figs. 4f, g; 5a, c; 6e-multipolar). However, the fact that unipolar, bipolar and multipolar cells are stained by horseradish peroxidase backfilling suggests an alternative conclusion [46].

The regional differences in the existence of small nerve cells may reflect functional zonation in the pineal organ (compare Figs. 3c and e; 4c and d). A zonation in the distribution pattern of nerve cells is described in the pineal organ of the pike, *Esox lucius*, [54, 55] where AChE-positive nerve cells are distributed in the rostral and proximal part of the pineal organ, whereas nerve cells are nearly absent and the photosensitive structures of the pinealocytes are reduced in the proximal part of the pineal end-vesicle. This kind of zonation does not seem to appear in other fish species [46, 51], but the regional differences in the number and shape of nerve cells are apparent. The small nerve cells, often localized at the basal side of the

FIG. 4. *Fugu pardale*. a, Sagittal section of the pineal end-vesicle. ×50. b and c, Large, intensely stained AChE-positive nerve cells scattered within the parenchyma of the pineal end-vesicle. They show sometimes a long and thick axon-like process (▲). b, ×475; c, ×120. d and e, In some regions of the pineal end-vesicle small nerve cells (⬆) are accumulated around a few large nerve cells. d, e, ×475. f-k. *Scomber japonica*. As in *Fugu*, intensely stained big nerve cells-sometimes multipolar in appearence- (▲) (f, g), sometimes with one axon-like process (⬆) (g, i, j). In some areas of the end-vesicle small, faintly stained small cells (⬆)are concentrated (k). f, g, h, ×475; i, ×260; j, k, ×475.

FIG. 5. a-m. Pineal complex of the deep-sea fish *Helicolenus hilgendorfi* (Cottidae, Teleostei). a-d, AChE-positive nerve cells in the pineal end-vesicle. A few number of intensely stained multipolar cells (⬆) (a, c) and a large number of faintly stained cells with one (▲) (a, b) or more (▲) (d) processes which characteristically bend and return near to the perikarya (*) (a, b). c, Pinealocytes project (⬆) their processes toward the multipolar cells. a, ×475; b, c, d, ×1200. e-j, Transverse (e, g-j) and cross sections (f) of the long, thin pineal stalk; most of the nerve cells are intensely stained (e, f). Some nerve cells contain a long, thick, axon-like process (▲) (h, i), but the process (⬆) which is sent by other nerve cells into the nerve fiber bundles, is thin (j). e, ×240; f, ×475; g, ×1200; h, ×475; i, ×1200; j, ×475. k and l, Parapineal organ (▲) with a large number of AChE-positive nerve cells (l) lateral to the pineal tract (⬆) in a cross section of the diencephalon. HN, habenular nucleus. k, ×120; l, ×475. m, Some nerve cells (*) are scattered along the parapineal tract which connects the parapineal organ with the brain (see also k). E, ependyma of the third ventricle.

FIG. 6. Pineal organ of the shark, *Triakis scyllia* (Selachii). a, The spherical end-vesicle is surrounded by the cartilaginous skull and ramified pigment cells. ×120. b-g, As in *Fugu* and *Scomber* large intensely stained AChE-positive nerve cells (b, c) and faintly stained small cells (⬆) (b, d) are distinguishable. The intensely stained cells show one (c), two (f) or more (e) processes. Some cells obviously send one process into the lumen of the end-vesicle (g). b, ×475; c, ×1200; d, e, f, ×475; g, ×1200. h-k, Nerve cells in the long, thin pineal stalk; they often send one process into the wide pineal lumen (⬆). h, ×475; i, ×600; j, k, ×475. l, The proximal part of the pineal stalk has a broad connection with the third ventricle (▲) at the rostral tip of the subcommissural organ (SCO). ×200. m, Single nerve cells (▲) are scattered along the pineal tract at the dorsal side of the subcommissural organ (SCO). ×1,200.

parenchyma (Figs. 3c; 4e, h, j, k), may be comparable to the pseudounipolar cells of the frog pineal complex. But it is possible that not all pineal nerve cells are AChE-positive. A population of GABA-immunoreactive neurons has been described in the rostral portion of the pineal end-vesicle of the rainbow trout. Additionally, GABA-positive cells have been found in the pineal stalk [50]. An interesting finding is the description of intrapineal nerve cells in the goldfish at the ultrastructural level, containing dense-cored vesicles, 80–160 nm in diameter, in their perikarya and their processes [52, 56]. These nerve cells are in contact with synaptic ribbon synapses of pinealocytes at their perikarya and their processes [56]. Dense-cored granules are known in neuropeptide-containing amacrine cells of the retina [49]. Intrapineal neurons containing granules may be an indication of the existence of interneurons.

A large number of pinealocytes related to one large nerve cell shown in the pineal end-vesicle of the goldfish [51] may be a sign of a higher photosensitivity in this part of the pineal organ in comparison to the pineal stalk. Generally, a greater number of nerve cells per area exist in the pineal stalk compared to the pineal end-vesicle [2, 51] (compare Fig. 3a and g; Fig. 5a and e). A small number of pinealocytes is related to each of these small nerve cells in the stalk area [51], and different types of photosensitive pinealocytes are described [36]. These findings make it likely that the small nerve cells conduct more specific light responses to the brain.

The pineal lumen of most of the lower vertebrates is in direct contact with the third ventricle (Fig. 6l); thus it contains cerebrospinal fluid (CSF). CSF-contacting neurons have been demonstrated in the shark pineal organ [4] (Fig. 6g-k) and further results are described for *Fugu niphobles* (Fig. 3f), *Scomber japonica* (Fig. 4j) and *Fugu pardale* (Fig. 4b, c). CSF-contacting neurons are also described in the cartilaginous fish, *Chimaera monstrosa*. The processes of pinealocytes terminate at their perikarya. The axons of these CSF-contacting neurons extend toward the pineal stalk. These neurons are comparable to the Landolt's bipolars of the retina [57] and their function is supposed to be an integration of changes in the composition of the pineal CSF and the photoreceptive activity of pinealocytes [36]. CSF-contacting neurons are also described in the rainbow trout by retrogradually labeling with horseradish peroxidase [58]. According to Ekstroem [58], they are second-order neurons receiving synaptic inputs from photosensitive pinealocytes and presynaptic to other neurons, and also send a long axon toward the brain. However, according to the same report, photosensitive pinealocytes also send a long process to the brain and other characteristics of CSF neurons are also true for pinealocytes [52, 59]. Further investigations seem to be necessary.

In general, the electrophysiological characteristics of the fish pineal complex are similar to those of the amphibians [19, 20, 25, 26, 48]. The photosensitive pinealocytes respond to light with a hyperpolarization whose amplitude correlated with intensity [60]. According to extracellular recordings, the trout pineal organ contains two kinds of photopigments [61]. The different response patterns of the pineal photoreceptors to bright flashes and to background illumination indicate the existence of several receptor types with rod- or cone-like characteristics in the teleostean pineal likely. Opsin immunoreactivity studies distinguished two different types of photoreceptors in the lamprey [see 36]. Visual adaptation is governed to a considerable extent by the photoreceptor cells themselves [62], but that does not exclude the involvement of pineal ganglion cells in the adaptation process [19]. A large number of photosensitive pinealocytes related to one nerve cell shown in the pineal end-vesicle of the goldfish are suggested to be an indication of a high degree of light sensitivity of this area [52]; in contrast, similar characteristics of the adaptation process in the dark of individual photosensitive pinealocytes and interneurons [62] supports the notion that the high light sensitivity of the pineal organ is not primarily due to a high convergence rate of photoreceptors onto centrally projecting ganglion cells, but mirrors the high sensitivity of individual pineal receptors [60]. Therefore, a clear discrepancy is obvious at moment between the morphological and electrophysiological findings in the explanation of the adaptation process.

In the goldfish, ganglion cells showed discharges

under conditions of steady illumination, both in dim and bright light; the response to light flashes was purely achromatic. The operating range of ganglion cells does not only depend on the absolute level of photoreceptor potentials: the most significant class of ganglion cells operates in a photopic-scotopic range with a nearly linear relationship between firing frequency and logarithm of background illumination, and the operating range of the other cell population is mainly at photopic levels of illumination [63, 64]. Possibly, both types of ganglion cells receive different inputs from other cells [63]. Morphological counting of 500 nerve cells and of only 310 nerve fibres in the pineal organ of the goldfish suggests the presence of interneurons [65]. Unfortunately only little is known about the possible presence of different neuronal populations in the pineal organ. In the rainbow trout, an intrapineal non-spiking interneuron exists that responds to light with a membrane hyperpolarization similar to that of photosensitive pinealocytes; there is a different decreasing response amplitude at higher light intensities between them. This may be due to a) interactions with other interneuronal types, b) a direct reciprocal innervation by ganglion cells or c) an effect of light adaptation by the previous light flash [60].

A small class of interneurons is described in *Phoxinus phoxinus* which exhibits a biphasic response pattern to light stimulation; the cells depolarize with dim light flashes and hyperpolarize with bright flashes. These cells do not show spike activity as ganglion cells, they have an atypical biphasic response pattern and they differ from photoreceptors in the voltage-intensity relation and likewise in the shape of the response. The spectral response curves peak at a wavelength different from that of photoreceptors, suggesting that this cell type receives complex receptor input. These cells provide further evidence of the presence of a differentiated network in the pineal tissue [66].

The nervous organization of the most primitive lamprey has been described by several investigators [26, 67–70]. Putative interneurons were not obseved in the lamprey pineal]71]. However, Morita *et al.* [72] describe a special type of nerve cells responding to light stimuli exclusively with off-discharges without spontaneous discharge.

Summarizing the morphological and electrophysiological results, there is evidence that the neuronal circuitry of the pineal complex in fish and amphibians exhibits a greater complexity than implied by the concept that signal transmission in the pineal organ is realized by a simple bineuronal pathway from photoreceptors to ganglion cells. But we are far from understanding of the functional meaning of the species-dependent and the intraspecific regional differences of the nervous organization of the pineal complex.

ACKNOWLEDGMENTS

The original results on the fish pineal complex were obtained during the stay by M. Ueck as a Visiting Professor of the Japan Society for the Promotion of Science at Misaki Marine Biological Station of the University of Tokyo (Head: Prof. H. Kobayashi). The authors thanks R. Liesner and A. Hach for their skillful technical assistance.

REFERENCES

1 Ruedeberg, C. (1969) Z. Zellforsch., **93**: 282–304.
2 Korf, H. W. (1974) Cell Tissue Res., *155*: 475–489.
3 Meiniel, A. and Collin J. P. (1971) Z. Zellforsch., **117**: 354–380.
4 Ueck, M. and Kobayashi H. (1979) Verh. Anat. Ges., **73**: 961–963.
5 Veen van Th. (1982) Cell Tissue Res., **222**: 433–444.
6 Studnička, F. K. (1905) Die Parietalorgane. In "Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere, 5A". Ed. by A. Oppel, Gustav Fischer, Jena, pp. 1–254.
7 Ueck, M. (1974) Fortschritte der Zoologie Bd. 22, Heft 2/3. Gustav Fischer, Stuttgart, pp. 167-203.
8 Ueck, M. (1982) Verh. Dtsch. Zool. Gesellsch. Hannover. Gustav Fischer, Stuttgart, pp. 61–80.
9 Vollrath, L. (1981) The pineal gland. In "Handbuch der mikroskopischen Anatomie des Menschen. Band 6/7". Ed. by A. Oksche and L. Vollrath, Springer-Verlag, Berlin/Heidelberg/New York, pp. 1–665.
10 Collin, J. P. and Oksche, A. (1981) In "The Pineal Gland: Anatomy and Biochemistry". Ed. by R. J. Reiter, CRC Press, Boca Raton, pp. 27–67.
11 Ueck, M., Vaupel-von Harnack, M. and Morita, Y. (1971) Z. Zellforsch, **116**: 250–274.
12 Eakin, R. M. and Westfall, J. A. (1961) Embryologia (Nagoya), **6**: 84–98.

13 Oksche, A. and Vaupel-von Harnack (1963) Z. Zellforsch., **59**: 230–288.
14 Oksche, A. and Vaupel-von Harnack, M. (1963) Z. Zellforsch., **59**: 582–614.
15 Holmgren, N. (1918/1919) Ark. Zool., **24**: 1–13.
16 Paul, E., Hartwig, H. G. and Oksche, A. (1971) Z. Zellforsch., **112**: 466–493.
17 Ueck, M. and Kobayashi, H. (1972) Z. Zellforsch., **129**: 140–160.
18 Wake, K., Ueck, M. and Oksche, A. (1974) Cell Tissue Res., **154**: 423–442.
19 Dodt, E., Ueck, M. and Oksche, A. (1971) In "J. E. Purkinje Centenary Symposium, Prague 1969". Ed. by V. Kruta, Universita Jena Evangelisty Purkyne, Brno, pp. 253–278.
20 Dodt, E. (1973) In "Handbook of Sensory Physiology". VII/3B, Ed. by R. Jung, Springer-Verlag, Berlin/Heidelberg/New York, pp. 113–140.
21 Morita, Y. and Dodt, E. (1965) Experientia (Basel), **21**: 221–222.
22 Hamasaki, D. I. and Esserman, L. (1976) J. Comp. Physiol., **109**: 279–285.
23 Meissl, H. and Donley, C. S. (1980) Vision Res., **20**: 379–383.
24 Hamasaki, D. I. (1970) Vision Res., **10**: 307–316.
25 Dodt, E. and Meissl, H. (1982) Experientia, **38**: 996–1000.
26 Morita, Y. (1975) In "Brain Endocrine Interaction II. The Ventricular System". Ed. by K. M. Knigge, D. E. Scott, H. Kobayashi and S. Ishii, Karger, Basel, pp. 376–387.
27 Hartwig, H. G. and Baumann, Ch. (1964) Vision Res., **14**: 597–598.
28 Dodt, E. and Morita, Y. (1964) Vision Res., **4**: 413–421.
29 Donley, C. S. and Meissl, H. (1979) Vision Res., **19**: 1343–1349.
30 Meissl, H. and George, S. R. (1984) Vision Res., **24**: 1727–1734.
31 Meissl, H. and George, S. R. (1985) Brain Res., **332**: 39–46.
32 Ehinger, B. (1982) Retina, **2**: 305–321.
33 Vigh, B. and Vigh-Teichmann, I. (1986) Arch. Histol. Japn., **49**: 495–518.
34 Vigh, B. and Vigh-Teichmann, I. (1981) Cell Tissue Res., **221**: 451–463.
35 Vigh, B., Vigh-Teichmann, I., Aros, B. and Oksche, A. (1985) Cell Tissue Res., **240**: 143–148.
36 Vigh, B. and Vigh-Teichmann, I. (1988) Pineal Research Reviews, **6**, Alan R. Liss, Inc., New York, pp. 1–65.
37 Ueno, S., Bambauer, H. J., Umar, H. and Ueck, M. (1984) Cell Tissue Res., **237**: 479–489.
38 Ueck, M., Umar, M., Umar, H. and Hach, A. (1987) In "Fundamentals and Clinics in Pineal Research". Ed. by G. P. Trentini, C. De Gaetani and P. Pévet, Sereno Symposia Publications, **44**, Raven Press, New York, pp. 53–56.
39 Scherer-Singler, U., Vincent, S. R., Kimura, H. and McGeer, E. G. (1983) J. Neuroscience Methods, **9**: 229–234.
40 Ueck, M., Sato, T., Ohba, S., Wake, K. and Kobayashi, H. (1989) In "Proceedings of the Internat. Symposium on Neurons and Paraneurons". Ed. by Fujita, Arch. Histol. Cytol. **52** (in press).
41 Sato, T. (1989) Arch. Histol. Cytol., (in press)
42 Boycott, B. B. (1988) Neuroscience Res., Suppl. **8**, Elsevier Scientific Publishers Ireland Ltd., pp. S97–S111.
43 Meiniel, A. (1980) Cell Tissue Res., **207**: 407–424.
44 Korf, H. W. and Ekstroem, P. (1987) In "Fundamentals and Clinics in Pineal Research". Ed. by G. P. Trentini, C. Gaetani, and P. Pévet, Sereno Symposia Publications, **44**, Raven Press, New York, pp. 35–47.
45 Eldred, W. D. and Nolte, J. (1981) J. Comp. Neurology, **203**: 269–295.
46 Ekstroem, P. and Korf, H. W. (1985) Cell Tissue Res., **240**: 693–700.
47 Ueck, M., Sato, T. and Ohba, S. (1989) Verh. Anat. Ges., **83**(Ulm), in press.
48 Meissl, H. and Dodt, E. (1981) In "The Pineal Organ: Photobiology-Biochronometry-Endocrinology". Ed. by A. Oksche and P. Pévet, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 61–80.
49 Rodieck, R. W. (1988) The primate retina. In "Comparative Primate Biology". **4**: Neuroscience, Alan R. Liss, Inc., New York, pp. 203–278.
50 Ekstroem, P., Veen van, Th., Bruun, A. and Ehinger, B. (1987) Cell Tissue Res., **250**: 87–92.
51 Wake, K. (1973) Z. Zellforsch., **145**: 287–298.
52 Ohba, S., Wake, K. and Ueck, M. (1979) Progress in Brain Res., **52**: 93–96.
53 Vigh-Teichmann, I., Korf, H.-W., Oksche, A. and Vigh, B. (1982) Cell Tissue Res., **227**: 351–369.
54 Falcón, J. (1979) Ann. Biol. Anim. Bioch. Biophys., **19**(2A): 445–465.
55 Falcón, J. and Meissl, H. (1981) J. Comp. Physiol., **144**: 127–137.
56 Ohba, S., Wake, K., Ohnishi, R. and Ueck, M. (1979) Verh. Anat. Ges., **73**: 953–959.
57 Vigh-Teichmann, I. and Vigh, B. (1987) In "Functional Morphology of Neuroendocrine Systems". Ed. by B. Scharrer, H.-W. Korf and H.-G. Hartwig, Springer-Verlag, Berlin/Heidelberg, p. 160.
58 Ekstroem, P. (1987) J. Neurosience, **7**: 987–995.
59 McNulty, J. A. (1980) Cell Tissue Res., **210**: 249–256.
60 Ekstroem, P. and Meissl, H. (1988) Neuroscience,

25: 1061–1070.

61 Meissl, H. and Ekstroem, P. (1988) Neuroscience, **25**: 1071–1076.

62 Meissl, H. and Ekstroem, P. (1988) Vision Res., **28**: 49–56.

63 Meissl, H., Nakamura, T. and Thiele, G. (1986) Comp. Biochem. Physiol., **84A**: 467–473.

64 Falcón, J. and Meissl, H. (1981) J. Comp. Physiol., **144**: 127–137.

65 McNulty, J. A. (1981) Canad. J. Zool., **59**: 1321–1325.

66 Nakamura, T., Thiele, G. and Meissl, H. (1986) J. Comp. Physiol., **A159**: 325–330.

67 Collin, J. P. (1969) J. Neuro-Visceral Relations, **31**: 308–333.

68 Meiniel, A. and Collin, J.-P. (1971) Z. Zellforsch, **117**: 354–380.

69 Cole, W. C. and Youson, J. H. (1982) Amer. J. Anat., **165**: 131–163.

70 Morita, Y. and Dodt, E. (1973) Nova Acta Leopoldina, **38**: 331–339.

71 Pu, G. A. and Dowling, J. E. (1981) J. Neurophysiol., **46**: 1018–1038.

72 Morita, Y., Tabata, M. and Tamotsu, S. (1985) Neurosci. Res. (Suppl.), **2**: S79–S88.